PB81-125676

Pentachlorophenol in the Environment Evidence for Its Origin from Commercial Pentachlorophenol by Negative Chemical Ionization Mass Spectrometry

Florida State Univ., Tallahassee

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

18 Jan 80

U.S. Department of Commerce
National Technical Information Service
NTIS.

ERL,GB- J0070

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-80-085	2. Journal Article	3. REC PB81-125676
4. TITLE AND SUBTITLE: Pentachlorophenol in the Environment. Evidence for Its Origin from Commerical Pentachlorophenol by Negative Chemical Ionization Mass Spectrometry		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Douglas W. Kuehl and Ralph C. Dougherty		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Same as Box # 12		10. PROGRAM ELEMENT NO. A2NN1E 11. CONTRACT/GRANT NO. Grant R8063340010
12. SPONSORING AGENCY NAME AND ADDRESS: U.S. Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Gulf Breeze, Florida 32561		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES: Ref: Analytical Chemistry Vol. 14 No. 4 April 1980 (447-449).

16. ABSTRACT

Commerical pentachlorophenol (PCP) contains significant quantities of tetrachlorophenol (TCP). The occurrence of TCP in environmental samples provides a chemical marker for PCP originating from commerical formulations. Negative chemical ionization mass spectrometry has been used to examine a commerical PCP formulation and a series of environmental and human samples. Tetrachlorophenol was determined by the ion current at m/z 229, tetrachlorophenoxide, and PCP was determined by the ion current at m/z 267, pentachlorophenoxide. The ion current at m/z 267 may include contributions from the oxygen/chloride exchange product of hexachlorobenzene, an environmental precursor of PCP. The ratio of PCP to TCP in Dowcide G-ST, a commerical PCP formulation was 2.5 ± 0.1. The ratio of m/z 267 to m/z 229 in a jellyfish, Mnemiopsis macrydi, from the Gulf of Mexico was 2.7 ± 0.1, in human seman it was 4.1 ± 0.1, and in human adipose tissue it was 15.5 ± 0.1. PCP in the seman was concentrated in the sperm cells by a factor of 9.

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Biochemistry Chromatography	Pentachlorophenol Tetrachlorophenol Negative Chemical Ionization Mass Spectrometry	06/A 06/T

18. DISTRIBUTION STATEMENT: Release to public	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS (This page): Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

Reprinted from ENVIRONMENTAL SCIENCE & TECHNOLOGY, Vol. 14, Page 447, April 1980
Copyright 1980 by the American Chemical Society and reprinted by permission of the copyright owner
600/J-80-085
Journal Article

Pentachlorophenol in the Environment. Evidence for Its Origin from Commercial Pentachlorophenol by Negative Chemical Ionization Mass Spectrometry

Douglas W. Kuehl[1] **and Ralph C. Dougherty***

Department of Chemistry, Florida State University, Tallahassee, Fla. 32306

■ Commercial pentachlorophenol (PCP) contains significant quantities of tetrachlorophenol (TCP). The occurrence of TCP in environmental samples provides a chemical marker for PCP originating from commercial formulations. Negative chemical ionization mass spectrometry has been used to examine a commercial PCP formulation and a series of environmental and human samples. Tetrachlorophenol was determined by the ion current at *m/z* 229, tetrachlorophenoxide, and PCP was determined by the ion current at *m/z* 267, pentachlorophenoxide. The ion current at *m/z* 267 may include contributions from the oxygen/chloride exchange product of hexachlorobenzene, an environmental precursor of PCP. The ratio of PCP to TCP in Dowcide G-ST, a commercial PCP formulation, was 2.5 ± 0.1. The ratio of *m/z* 267 to *m/z* 229 in a jellyfish, *Mnemiopsis macrydi*, from the Gulf of Mexico was 2.7 ± 0.1, in human semen it was 4.1 ± 0.1, and in human adipose tissue it was 15.5 ± 0.1. PCP in the semen samples was concentrated in the sperm cells by a factor of 9.

The widespread contamination of human populations in the United States (*1, 2*), western Europe (*3*), and Japan (*4*) with pentachlorophenol at concentrations roughly 10^{-3} of the lethal dose for pentachlorophenol in humans (*5*) has been the subject of recent discussion (*5, 6*). Moreover, it has been suggested that pentachlorophenol contamination of the human population may be due to the metabolism of hexachlorobenzene (*6*), since pentachlorophenol is a metabolite of hexachlorobenzene in mammals (*7*). There has also been an unsupported suggestion that the presence of pentachlorophenol in humans may be an expression of a natural phenomenon, i.e., that pentachlorophenol is a natural metabolite of some organisms (*6*).

In order to obtain information about the origin of pentachlorophenol in the environment, we have applied negative chemical ionization (NCI) mass spectrometric screening techniques (*8*) to a commercial sample of pentachlorophenol (Dowcide G-ST) and purified samples of environmental substrates. The basic strategy in this investigation derives from the fact that commercial pentachlorophenol is very generally contaminated with tetrachlorophenol isomers. The tetrachlorophenol isomers will migrate with pentachlorophenol in the environment and, although the rates of metabolism and excretion of tetrachlorophenol isomers may be different from that of pentachlorophenol, the presence of these compounds provides a marker for the origins of pentachlorophenol in the samples.

Experimental

Mass Spectrometry. Negative chemical ionization mass spectra were obtained by direct probe introduction, using an AEI MS-902 mass spectrometer equipped with an SRIC chemical ionization source. Ionization was initiated with 470 V-e with a regulated emission current of 0.25 mA. Source pressures were monitored by use of a MKS Betatron which was operated at the source potential (−8 kV) and isolated from the earth. Spectral quality methylene chloride was distilled prior to use, and Matheson research grade isobutane (1:1), with the addition of 2% oxygen, was used as the reagent gas. Spectra were obtained with a source temperature of 150 °C and source pressures of 0.5 Torr, 30 s after sample introduction.

Gas chromatographic–mass spectrometric data were obtained using a Varian MAT CH-5 MS/Varian Aerograph 1700 GC equipped with a 6 ft × ⅛ in. glass column packed with 3% OV-101 on 80/100 mesh Gas-Chrom Q. A Finnigan INCOS data system was used to record spectra.

Derivatization. Phenols in Dowcide G-ST were derivatized using a 50× excess of tri-sil-BSA (Pierce Chemical Co.) at room temperature.

Cleanup Procedure. The primary cleanup step used in these analyses was steam distillation. The steam distillation head used in these procedures was a slightly modified (*9*) version of that described by Veith and Kiwus (*10*).

In a typical experiment, 250 mg of fresh adipose tissue was macerated with 150 mL of 10% sulfuric acid in a Vertis blender. The homogenate was warmed at 80 °C for 40 minutes and then steam distilled into 2 mL of distilled-in-glass 2,2,4-trimethylpentane (Burdick and Jackson). After 1 h of steam distillation, the aqueous and "isooctane" layers were removed from the steam head and separated by use of a Pasteur pipet. Samples of the organic layer were concentrated using a Snyder column. The concentrate was placed on a quartz probe tip and introduced directly into the NCI mass spectrometer. The seminal fluid (2.0 mL) and jellyfish (100 g) samples were processed in a similar manner.

Results and Discussion

Environmental analytical chemistry requires separation of the components of interest from the matrix in which they are found and the subsequent analysis of those components by an appropriate technique. The current analytical protocol calls for utilization of steam distillation, followed by application of a very specific detector: a negative chemical ionization (NCI) mass spectrometer. This analysis system was chosen because NCI mass spectrometry is uniquely suited for screening partially cleaned-up samples of environmental substrates for contamination with toxic substances. NCI mass spectra of toxic materials can generally be obtained with low nanogram sensitivities compared with a sensitivity to the biomolecules that usually dominate environmental samples that is often three or four orders of magnitude lower. That is, 10 μg of potentially interfering material would be required to obtain a reproducible spectrum at a low nanogram sensitivity for the toxicant in question. The reason for this difference in sensitivity is easy to understand on the basis of two observations: (1) The biomolecules are highly reduced with the exception of free carboxylic acids and some of the prosthetic groups from the electron-transport chain. These molecules, which have negative electron affinities and only very weakly attach gas-phase nucleophiles, are virtually transparent to negative chemical ionization. (2) A carcinogenic substance can be very generally classed as an oxidizing agent, an alkylating agent, or a molecule that can be converted readily into an alkylating agent. These molecules would be highly responsive to NCI because they generally have positive electron affinities and attach gas-phase nucleophiles to form stable complexes.

The analytical application of NCI mass spectrometry is

[1] On Interagency Personnel Agreement assignment from the U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minn. 55804.

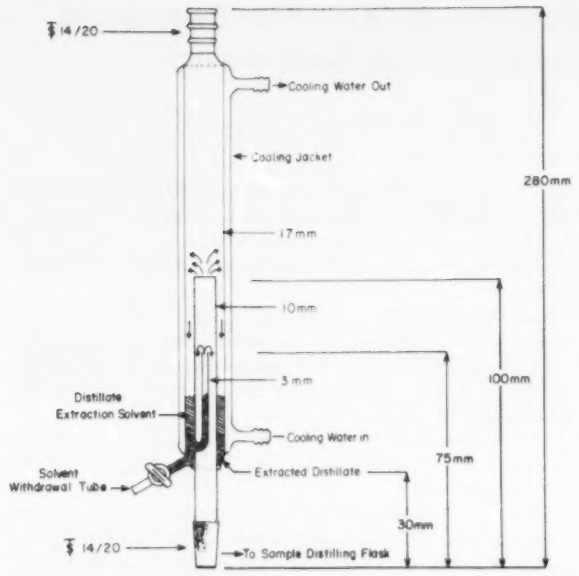

Figure 1. Steam distillation head used for sample cleanup showing path of water vapors and construction dimensions

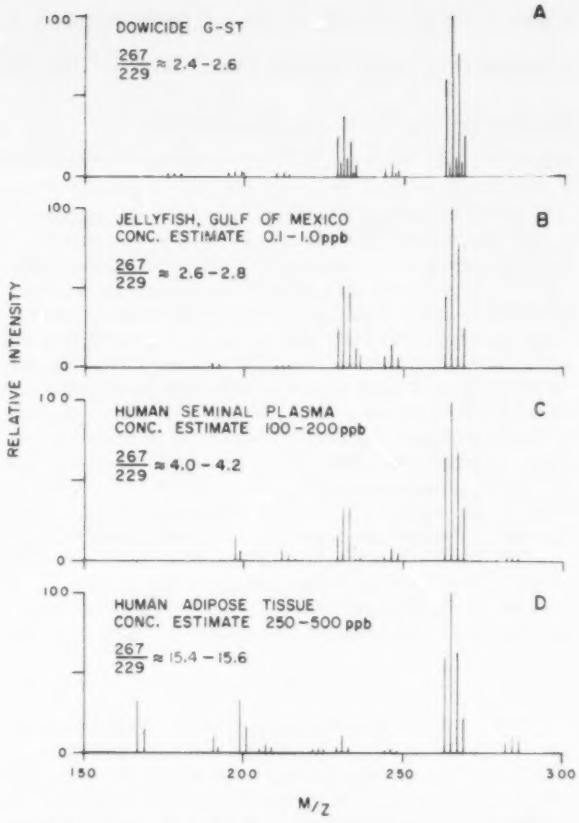

Figure 2. Negative mass defect ions in the isobutane–oxygen NCI mass spectra of (a) Dowcide G-ST; (b) steam distillate of a Gulf coast jellyfish; (c) steam distillate of human seminal fluid; (d) steam distillate of human adipose tissue (see Experimental section for details of NCI conditions)

broader but quite similar to that of gas chromatography with electron-capture detection. Compounds that give intense electron-capture responses will virtually always be sensitive to NCI mass spectra. Moreover, mass resolution in NCI spectra serves the same function as time resolution in gas chromatography. However, by using a high-resolution mass spectrometer it is possible to obtain mass resolution of the order of one part in 10^4, which is considerably higher than is available with a conventional gas chromatograph. The reduction in chemical noise by mass analysis also contributes to the lower detection limit for the NCI system as compared to gas chromatography.

NCI mass spectra are obtained by operating a chemical ionization mass spectrometer (ion source pressure ca. 1 Torr) in the negative ion mode. Under these conditions there are five major negative ion forming reactions that occur, assuming that the ion source contains oxygen and organochlorine compounds. These reactions are the following:

$$M + e_s^- \rightarrow M^{*-} \cdot \xrightarrow{N} M^- \cdot \quad (1)$$

$$M + Cl^- \rightarrow (M + Cl)^- \quad (2)$$

$$M + Cl^- \rightarrow (M - H)^- + HCl \quad (3)$$

$$M + O_2^- \cdot \rightarrow (M - Cl + O)^- + ClO \cdot \quad (4)$$

$$M + e_s^- \rightarrow A^- + B \cdot \quad (5)$$

The dominant ion in the NCI mass spectra of most organic polychlorides is $(M \pm Cl)^-$. However, the predominant mode of ionization for pentachlorophenol (PCP) is an acid–base exchange reaction (3) or a disassociative electron capture (5), which can give $(M - H)^-$ ions.

Steam distillation has been shown to be a very efficient purification technique (*10, 11*) due to the difference in vapor pressure of large biological molecules compared to xenobiotic chemicals. Carbohydrates, proteins, and neutral lipids are "nonvolatile" with steam, while many toxic substances, such as pentachlorophenol, have appreciable vapor pressures. The apparatus used is a continuous liquid–liquid extraction steam distillation (Figure 1) that offers an exceptionally simple one-step cleanup procedure. Sample recoveries for polychlorinated aromatic hydrocarbons (*10*) and polynuclear aromatic hydrocarbons (*11*) have consistently exceeded 90%. The recovery of pentachlorophenol from water, pH 7, at the 50-ppb level has ranged from 96 to 98%.

In addition to xenobiotic chemicals, low molecular weight plant phenolics, alkaloids, and free fatty acids will also steam distill. Since free fatty acids have a high sensitivity for chloride attachment (*12*), they are the most consistent interference in NCI screening of environmental samples. In the current study, the complexity of NCI spectra due to the free fatty acids and the presence of large numbers of xenobiotic chemicals (*13*) requires that the spectra include only the negative mass defect ions of the ^{12}C isotopic peaks.

Figure 2 presents the m/z 150 to m/z 300 segments of the NCI mass spectra of Dowcide G-ST and concentrated steam distillates of the jellyfish, adipose tissue, and seminal fluid. The jellyfish, *Mnemiopsis macrydi*, were collected on the shores of St. George Island off the coast of the North Florida panhandle in the Gulf of Mexico. The adipose tissues are autopsy samples from the School of Medicine, University of Florida, Gainsville, Fla., and the seminal fluid samples were collected from volunteer student donors at Florida State University. The three major ion clusters at m/z 229, 244, and 263 in the spectra of Dowcide G-ST represent, respectively, tetrachlorophenoxide, tetrachlorobenzoquinone (ortho and para isomers), and pentachlorophenoxide. Tetrachlorophenoxide and pentachlorophenoxide arise from the loss of hydrogen from tetrachlorophenol and pentachlorophenol, and the tetrachlorobenzoquinone anion arises from a chloride–oxygen exchange reaction of pentachlorophenoxide. The presence of isomers of tetrachlorophenol in Dowcide G-ST has been confirmed by electron-impact GC–MS.

The chloride isotope distributions in the tetrachlorophe-

noxide ions m/z 229, 231, and 233 in the environmental samples appear distorted in Figure 2 for two reasons. First, the negative mass defect at 233 appears as part of an incompletely resolved isobar due to the chloride adduct of dodecenoic acid, and, secondly, the ions at m/z 231 and 233 are distorted because of ion current due to the chloride adduct of trichlorophenol. The isotope distribution of the pentachlorophenoxide ions m/z 263, 265, and 267 can also be disturbed because of an unresolved isobar at m/z 263 due to the chloride adduct of myristic acid. During a recent study of human adipose tissue and human seminal fluid (*9*), it was found that m/z 229 and m/z 267 were free of interferences and that their ratio can represent the ratio of tetrachlorophenoxide to pentachlorophenoxide in biological samples.

The NCI mass spectrum of hexachlorobenzene obtained with isobutane and oxygen as reagent gases consists of pentachlorophenoxide (m/z 265, 100%), the molecule anion (m/z 282, 83%), and tetrachlorobenzoquinone (m/z 244, <0.01%). The increasing ratios of pentachlorophenoxide to tetrachlorophenoxide in Figures 2c and 2d as compared to Figures 2a and 2b probably reflect a contribution from hexachlorobenzene to the pentachlorophenoxide ion current. It is interesting to note that the ratios of the ion currents at m/z 229 and 244 were 5.2, 4.5, 5.3, and 5.0 for the spectra presented in Figures 2a and 2d, respectively. The ratio of those two ion intensities for this series of experiments is therefore considered constant considering the signal-to-noise ratio for these high sensitivity measurements and the complications associated with partially resolved positive mass defect isobars. This result suggests that the pentachlorophenol in the three biological samples examined had commercial pentachlorophenol as its direct origin. Pentachlorophenol-treated wood products are a likely source of the contamination of the human samples, while the jellyfish were probably contaminated as a result of the use of PCP as a slimicide in the drilling mud of oil drilling rigs in the Gulf.

The concentration estimates given in Figure 2 were obtained by measuring the sensitivity for detection of pentachlorophenol at a signal-to-noise ratio of 2:1; this figure varied with ion source condition, but was generally in the range of 10 ng. Concentrations were then estimated from a knowledge of the sample size and the signal intensity, assuming a linear response to the detector.

In order to determine the partition of pentachlorophenol between seminal fluid and sperm cells, the cells were concentrated by centrifugation and the cells and the supernatant fluids were analyzed separately for pentachlorophenol. For a 2-mL sample of seminal fluid containing 8.5×10^6 cells per mL, the mass of the cells was 0.08 g and the mass of the seminal fluid was 1.93 g. It was observed that 40% of the pentachlorophenol was bound to the cellular material, which comprised only 4% of the total sample mass. This suggests that pentachlorophenol is selectively concentrated by the cellular material. Pentachlorophenol has been identified in 50 of 50 seminal fluid samples analyzed in a random sampling of university students and 12 of 12 human adipose tissue samples (*9*). Since pentachlorophenol is mildly mutagenic (*14*), this result is a cause for concern from an environmental point of view. The selection of seminal fluid as a substrate for evaluation of contamination of the biosphere with pentachlorophenol stems from the fact that there has been an apparent decrease in male fertility potential in the United States over the last 30 years, as evidenced by declining sperm density (*9*, *15*).

The data above suggest that a simple one-step cleanup by steam distillation and analysis by negative chemical ionization mass spectrometry result in highly selective and sensitive examination of environmental samples for xenobiotic chemicals. More specifically, the three biological substrates that were examined were found to contain pentachlorophenol that had its origins in commercial pentachlorophenol, and large numbers of samples of these substrate types were generally found to contain pentachlorophenol. These results demonstrate a cause for concern and suggest that the utility of pentachlorophenol relative to environmental health should be reviewed.

Literature Cited

(1) Bevenue, A., Beckman, H., *Bull. Environ. Contam. Toxicol.*, **2**, 319 (1967).
(2) Dougherty, R. C., Piotrowska, K., *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1777 (1976).
(3) Carnmer, M. F., Freal, J., *Life Sci.*, **9**, 121 (1970).
(4) Akisada, T., *Bunseki Kagaku*, **14**, 101 (1965).
(5) Dougherty, R. C., in "Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology", Raol, K. R., Ed., Plenum, New York, 1978, p 351.
(6) Detrick, R. S., *For. Prod. J.*, **27**, 13 (1977).
(7) Yang, R. S., Coulston, H. F., Goldberg, L., *J. Assoc. Off. Anal. Chem.*, **58**, 1197 (1975).
(8) Dougherty, R.C., Hett, E. A., in ref *5*, p 339.
(9) Dougherty, R. C., Whitaker, M. J., Smith, L. M., Stalling, D. L., Kuehl, D. W., *Environ. Health Perspec.*, in press.
(10) Veith, G. D., Kiwus, L. M., *Bull. Environ. Contam. Toxicol.*, **17**, 631 (1977).
(11) Kuehl, D. W., Whitaker, M. J., Wander, J., and Dougherty, R. C., *Anal. Chem.*, in press.
(12) Tannenbaum, H. P., Roberts, J. D., Dougherty, R. C., *Anal. Chem.*, **47**, 49 (1975).
(13) Dougherty, R. C., Bergner, N., Levonowich, P., Roberts, J. D., *Adv. Mass Spectrom. Biochem. Med.*, **1**, 181 (1976).
(14) Fahrig, R., *IARC Sci. Publ.*, **No. 10**, 161 (1975).
(15) Nelson, C. N. K., Bunge, R. D., *Fertil. Steril.*, **25**, 503 (1974).

Received for review September 18, 1979. Accepted January 18, 1980. This work was supported by grants from the U.S. Environmental Protection Agency and the National Institutes of Health.